

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No.	: 10/629,895	Confirmation No.: 8585
Applicant	: John J. ROSSI <i>et al.</i>	
Filed	: 30 July 2003	
TC/A.U.	: 1635	
Examiner	: Brian A. WHITEMAN	
Attorney Docket No.	: 1954-413	
Customer No.	: 6449	

Director of the United States Patent
and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

RULE 132 DECLARATION

Dear Sir:

I, John J. Rossi, hereby declare as follows:

1. I am an inventor of the above-identified application. I am also an inventor of the subject matter disclosed in Rossi et al. (US 6,100,087) and in Rossi et al. (US 6,995,258)
2. My educational background and experience are shown in my *curriculum vitae* attached hereto as Exhibit A.
3. I have read the Office Action dated January 5, 2009. It is my understanding that the Examiner has rejected claims 1, 2 and 11-16 under 35 U.S.C. § 103(a) as being obvious over Agami et al. (US 7,241,618) taken with Doglio et al. (US 5,837,503) in further view of either Yu et al. (*Proc Natl Acad Sci USA* **99**:6047-6052, 2002) or Ambros (*Cell* **107**:823-826, 2001). It is my understanding that the Examiner cites Agami et al. for its disclosure of an expression cassette comprising an adenoviral VA1 promoter operably linked to an siRNA molecule which could be an shRNA molecule and its disclosure that the siRNA molecule is a substrate for Dicer. It is my understanding that the Examiner cites Doglio et al. for its disclosure of an expression cassette in which an nucleic acid encoding either an antisense oligonucleotide or a ribozyme has been inserted between or outside the boxes A and B constituting the promoter of the VA1 gene or into the VA1 gene. It is my understanding that the Examiner cites Yu et al. for its disclosure of an RNA pol III

vector comprising shRNA which can inhibit expression in mammalian cells and cites Ambros for its disclosure of miRNA. It is my understanding that it is the Examiner's position that it would have been *prima facie* obvious to combine the teachings of these references to produce an expression vector comprising an adenoviral VA1 promoter wherein an RNAi molecule is contained within a non-essential stem region of the promoter or coding region of the VA1 gene. The Examiner contends that a skilled artisan would have been motivated to combine these teachings to avoid reducing the activity of the promoter or to successfully express the RNAi molecule in cells. I disagree.

4. It is also my understanding that the Examiner has rejected claims 1, 2 and 3 under 35 U.S.C. § 103(a) as being obvious over Agami et al. taken with Doglio et al. in further view of either Yu et al. or Ambros and in further view of Cagnon et al. (*Antisense Nucl Acid Drug Dev* 10:251-261, 2000). It is my understanding that the Examiner cites Agami et al., Doglio et al., Yu et al. and Ambros as discussed in Paragraph 3. In it my understanding that the Examiner cites Cagnon et al. for its disclosure of inserting an RNAi molecule (i.e., ribozyme) using a filled in NotI site that was ligated into the BstEII cleaved, filled in vector. It is my understanding that the Examiner then concludes that it would have been *prima facie* obvious to produce an expression cassette in which the non-essential region contains a BstEII site. I disagree.

5. It is also my understanding that the Examiner has rejected claims 1, 5 and 6 under 35 U.S.C. § 103(a) as being obvious over Agami et al. taken with Doglio et al. in further view of either Yu et al. or Ambros and in further view of Lorens (US 2004/0005593). It is my understanding that the Examiner cites Agami et al., Doglio et al., Yu et al. and Ambros as discussed in Paragraph 3. It is my understanding that the Examiner cites Lorens for its disclosure of an RNAi molecule having a loop containing at least 6 nucleotides. It is my understanding that the Examiner then concludes that it would have been *prima facie* obvious to produce an expression cassette comprising an adenoviral VA promoter in which an RNAi molecule comprises a loop containing about 8 nucleotides. I disagree.

6. It is also my understanding that the Examiner has rejected claims 1, 2 and 11-16 under the judicially created doctrine of obviousness-type double patenting over claims 1 and 7-9 of Rossi et

Rossi et al. (US 6,995,258) taken with Agami et al. and Doglio et al. further in view of Zeng et al. (*Mol Cell* 9:1327-33, 2002) and Yu et al. It is my understanding that the Examiner cites Agami et al. a, Doglio, and Yu et al. as discussed in Paragraph 3. It is my understanding that the Examiner cites Zeng for its disclosure of miRNAs. It is my understanding that the Examiner concludes that that it would have been *prima facie* obvious to combine the teachings of these references to produce an expression cassette comprising an RNAi molecule operatively linked to adenoviral VA1 promoter wherein the RNAi is shRNA or precursor miRNA. I disagree.

7. It is also my understanding that the Examiner has rejected claims 1, 5 and 6 under the judicially created doctrine of obviousness-type double patenting over claims 1 and 7-9 of Rossi et al. '258 taken with Agami et al. and Doglio et al. further in view of Zeng et al. in further view of Lorens. It is my understanding that the Examiner cites Agami et al. and Doglio et al. as discussed in Paragraph 3, cites Zeng et al. as discussed in Paragraph 6 and cites Lorens as discussed in Paragraph 5. It is my understanding that the Examiner concludes that that it would have been *prima facie* obvious to combine the teachings of these references to produce an expression cassette comprising an RNAi molecule operatively linked to adenoviral VA1 promoter wherein the RNAi has a loop containing 8 nucleotides. I disagree.

8. It is also my understanding that the Examiner has rejected claims 1, 2 and 3 for obviousness-type double patenting over claims 1 and 7-9 of Rossi et al. '258 with taken with Agami et al., Doglio et al. and Zeng et al. or Yu et al. in further view of Cagnon et al. It is my understanding that the Examiner cites Agami et al., Doglio et al. and Yu et al. as discussed in Paragraph 3, cites Zeng et al. as discussed in Paragraph 6 and cites Cagnon et al. as discussed in Paragraph 4. It is my understanding that the Examiner concludes that that it would have been *prima facie* obvious to combine the teachings of these references to produce an expression cassette comprising an RNAi molecule operatively linked to adenoviral VA1 promoter wherein the non-essential stem region contains a BstEII site. I disagree.

9. It is further my understanding that the Examiner contends with respect to each of the above rejections that arguments that were made in the Amendment dated 2 October 2008 are not persuasive. The Examiner contends that a skilled artisan would have been motivated to combine the

prior art to successfully express an RNAi molecule in a cell. The Examiner further contends that a skilled artisan understands that RNA transcripts are cleaved citing Agami et al. and Sharp et al. (*Genes & Development* **15**:485-490, 2001). The Examiner contends that it is not required that the RNAi molecule be cleaved out of the VA1 transcript because the transcript would be cleaved and become a substrate for Dicer as taught by Agami et al. and Sharp et al.

10. I have read and understand the Amendment that is being file concurrently herewith.

11. It is my understanding that the claims as amended in this Amendment are directed to an expression cassette and a mammalian cell into which the expression cassette has been introduced. The expression cassette comprises an adenoviral VA1 gene and a nucleic acid encoding an interfering RNA (RNAi) molecule. The adenoviral VA1 gene comprises the adenoviral VA1 promoter and a coding sequence for the VA1 RNA. The nucleic acid is inserted within the adenoviral VA1 coding sequence. The nucleic acid encodes a hairpin siRNA (shRNA) or a precursor microRNA (precursor miRNA). The RNAi molecule is a substrate for Dicer (i) upon expression of the VA1 RNA which includes the RNAi molecule and (ii) after the RNAi molecule is processed out of the VA1 RNA. That is, in order to be a substrate for Dicer, the RNAi molecule must be removed from the VA1 RNA. Amended claims 1 and 11 state that the RNAi molecule is processed from the VA1 RNA to become a substrate for Dicer.

12. It is my opinion that the Examiner's interpretation of Agami et al. and Sharp et al. with respect to the VA1 transcript and substrates for Dicer is incorrect. Specifically, columns 1-3 of Agami et al. describe the general background of small interfering RNA (siRNA). Agami et al. discloses the discovery that double stranded RNAs (dsRNAs) are capable of inhibiting the expression of target genes. As described in Agami et al., dsRNA added to cells is processed into duplexes of from 21 to 25 nucleotides in length, which in *Drosopholia* is performed by an enzyme termed Dicer. See, column 1, line 63 – column 2, line 23. The strand of the dsRNA complementary to the target mRNA hybridizes to the target mRNA and the target mRNA is then cleaved. See, column 2, lines 25-32. The cleavage that is described in Agami et al. relates to (i) the cleavage of the dsRNA by Dicer to form an active siRNA and (ii) the cleavage of the target transcript upon hybridization of the active siRNA to the target transcript. There is no disclosure in Agami et al.

concerning the adenoviral VA1 transcript. Nor is there any disclosure in Agami et al. that the VA1 transcript is cleaved. There is no disclosure in Agami et al. that the VA1 transcript would be cleaved and become a substrate for Dicer.

13. Similarly, Sharp et al., which is a review article on RNA interference, discloses that dsRNA added to extracts of *Drosophila* embryos were cleaved to 21 to 23 nucleotide dsRNAs. These smaller dsRNAs led to the cleavage of mRNA of the target gene upon hybridization of the complementary strand of the smaller dsRNA to the target mRNA. See, the paragraph bridging the left and right columns on page 385. The complex that generates the siRNA from short dsRNA recognizes the 3' termini of the duplex and internal cleavage occurs at a distance of approximately 22 nucleotides. The complex with siRNA then associates with the target mRNA leading to cleavage of the target mRNA. See, "Genesis of RNAi" on page 486. There is no disclosure in Sharp et al. concerning the adenoviral VA1 transcript. Nor is there any disclosure in Sharp et al. that the VA1 transcript is cleaved. There is no disclosure in Sharp et al. that the VA1 transcript would be cleaved and become a substrate for Dicer.

14. Thus, the cleavage that is described in Agami et al. and Sharp et al. is the cleavage of dsRNA introduced into a cell by Dicer to form an active siRNA which then hybridizes to the target mRNA leading to cleavage of the target mRNA. The target mRNA, i.e., the target RNA transcript, is only cleaved if there is an active siRNA present. The active siRNA is only present if the dsRNA is accessible to be cleaved by Dicer and cleaved by Dicer. There is no disclosure in Agami et al. and Sharp et al. that RNA transcripts are cleaved in the absence of siRNA molecules. Thus, a skilled artisan only understands that an RNA transcript is cleaved if an active siRNA molecule targeted to the RNA transcript is present in the cell. Not only is there no disclosure that RNA transcripts are cleaved in the absence of siRNA molecules, there is no disclosure in either Agami et al. or Sharp et al. that the adenoviral VA1 RNA, i.e., adenoviral VA1, transcript is cleaved in a cell. There is no disclosure in either Agami et al. or Sharp et al. that the adenoviral VA1 transcript would be cleaved in a cell and become a substrate for Dicer.

15. In fact, it is my opinion that the prior art, including prior art cited by the Examiner, specifically teaches that the adenoviral VA1 transcript is not cleaved in a cell. Specifically, Doglio

et al., Rossi et al. (US 6,100,087) and Cagnon et al. each teach that the adenoviral VA1 transcript containing an inhibitory RNA molecule is not cleaved in a cell. Doglio et al. teaches the introduction of a nucleic acid sequence encoding an inhibitory RNA, specifically an antisense oligonucleotide or a ribozyme, into the adenoviral VA1 gene which comprises the adenoviral VA1 promoter and coding sequence for the VA1 RNA. The coding sequence for the antisense oligonucleotide or ribozyme is inserted into the VA1 gene between or outside of the promoter A and B boxes. See, column 3, lines 3-9 and column 5, lines 11-13. Doglio et al. teaches that the antisense oligonucleotide or ribozyme is an "extrusion" relative to the VA1 transcript and is able to access the mRNA. See, column 4, lines 54-60. Thus, Doglio et al. teaches that the inhibitory RNA (i.e., antisense oligonucleotide or ribozyme) is active within the VA1 transcript. That is, Doglio et al. teaches that the inhibitory RNA molecule does not need to be cleaved from the VA1 transcript in order to be active for inactivating or cleaving a target gene transcript. Furthermore, Doglio et al. shows that the VA1 transcript containing the inhibitory RNA molecule is not cleaved in a cell. Specifically, Figure 3 and 8 show that RNA polymerase III transcription of the expression constructs produces an RNA transcript containing the inhibitory RNA molecule and the level of expression is not affected. See, Figures 3 and 8 and the description at column 9, lines 38-40 and lines 56-60, column 10, line 57 – column 11, line 8 and column 12, lines 42-61. Figures 3 and 8 do not show any cleavage of the VA1 transcript containing the inhibitory RNA molecule. Thus, Doglio et al. teaches that the VA1 transcript containing an inhibitory RNA molecule is not cleaved in a cell.

16. Rossi et al. ('087) teaches the introduction of a nucleic acid sequence encoding an inhibitory RNA, specifically a ribozyme, into the adenoviral VA1 gene which comprises the adenoviral VA1 promoter and coding sequence for the VA1 RNA. Rossi et al. ('087) teaches that the VA1 transcript is very long-lived in the cytoplasm. See, column 5, lines 20-22. The coding sequence for the ribozyme is inserted into the VA1 gene outside of the promoter A and B boxes. See, Figure 9. Rossi et al. ('087) teaches that the ribozyme is located at the top of the stem loop structure relative to the VA1 transcript and is active. See, column 5, lines 22-33. Thus, Rossi et al. ('087) teaches that the inhibitory RNA (i.e., ribozyme) is active within the VA1 transcript. That is, Rossi et al. ('087) teaches that the inhibitory RNA molecule does not need to be cleaved from the

VA1 transcript in order to be active for inactivating or cleaving a target gene transcript. Furthermore, Rossi et al. ('087) shows that the VA1 transcript containing the inhibitory RNA molecule is not cleaved in a cell. Specifically, Figure 6 and 7 show that RNA polymerase III transcription of the expression constructs produces an RNA transcript containing the inhibitory RNA molecule. See, Figures 6 and 7 and the description at column 1, lines 60-64 and column 6, lines 20-441. Figures 6 and 7 do not show any cleavage of the VA1 transcript containing the inhibitory RNA molecule. Thus, Rossi et al. ('087) teaches that the VA1 transcript containing an inhibitory RNA molecule is not cleaved in a cell.

17. Cagnon et al. teaches the introduction of a nucleic acid sequence encoding an inhibitory RNA, specifically a ribozyme, into the adenoviral VA1 gene which comprises the adenoviral VA1 promoter and coding sequence for the VA1 RNA. The coding sequence for the ribozyme is inserted into the VA1 gene outside of the promoter A and B boxes. See, Figure 3A. Cagnon et al. teaches that the ribozyme is located at the top of the stem loop structure relative to the VA1 transcript and is active. See, Figures 3B and Figure 4A and page 255. Thus, Cagnon et al. teaches that the inhibitory RNA (i.e., ribozyme) is active within the VA1 transcript. That is, Cagnon et al. teaches that the inhibitory RNA molecule does not need to be cleaved from the VA1 transcript in order to be active for inactivating or cleaving a target gene transcript. Furthermore, Cagnon et al. shows that the VA1 transcript containing the inhibitory RNA molecule is not cleaved in a cell. Specifically, Figures 3D and 3E show that RNA polymerase III transcription of the expression constructs produces an RNA transcript containing the inhibitory RNA molecule. See, 3D and 3E and the description at page 255. Figures 3D and 3E do not show any cleavage of the VA1 transcript containing the inhibitory RNA molecule. Thus, Cagnon et al. teaches that the VA1 transcript containing an inhibitory RNA molecule is not cleaved in a cell.

18. The above analysis of Doglio et al., Rossi et al. ('087) and Cagnon et al. clearly demonstrates that the prior art teaches that the adenoviral VA1 transcript modified to contain an inhibitory RNA molecule, i.e., an antisense oligonucleotide or a ribozyme, is not cleaved in a cell. These references further show that the inhibitory molecule is active without being cleaved from the VA1 transcript, i.e., the inhibitory molecule is active without being processed from the VA1

transcript. Thus, the active inhibitory RNA molecule produced by the cited Doglio et al. and Cagnon et al. is part of the VA1 transcript. The successful production of the inhibitory RNA molecule of Doglio et al. and Cagnon et al. in a cell is only as part of the VA1 transcript. There is no teaching in the prior art that the inhibitory molecule *per se* is produced other than as part of the VA1 transcript.

19. As discussed above in Paragraphs 12-14, Agami et al. and Sharp et al. teach that exogenous dsRNA introduced into a cell is cleaved by Dicer to produce siRNA molecules of about 21-25 nucleotides. The dsRNA must be accessible to Dicer and its cell machinery in order for it to be cleaved into the appropriate sized siRNA molecule. Agami et al. and Sharp et al. further teach that the siRNA molecule then hybridizes with the target mRNA transcript resulting in cleavage of the target mRNA transcript. It is only upon hybridization of the siRNA molecule with the target mRNA transcript that cleavage of the transcript occurs. The mRNA transcript is not cleaved in the absence of an siRNA molecule targeted to the RNA transcript. The cited Ambros reference teaches that a similar mechanism exists for microRNA (miRNA) in which the precursor miRNA is cleaved by Dicer to produce an miRNA molecule of about 22 nucleotides, which is the active molecule. Thus, it is my opinion that the skilled artisan understands that RNA transcripts are not randomly cleaved without the appropriate cellular mechanisms.

20. As discussed above in Paragraphs 15-18, Doglio et al., Rossi et al. ('087) and Cagnon et al. teach that the VA1 transcript containing an inserted inhibitory RNA molecule is not cleaved in a cell. The teachings of these references are consistent with the teachings of Agami et al. and Sharp et al. that RNA transcripts are not cleaved in the absence of siRNA molecules. Thus, it is my opinion that the skilled artisan understands that the VA1 transcript containing an inhibitory RNA molecule is not cleaved in a cell. Because a skilled artisan understands that such a VA1 transcript is not cleaved, it is also my opinion that the prior art teaches away from the present invention.

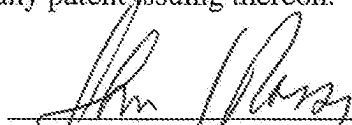
21. In order for the RNAi molecule, i.e., the shRNA or precursor miRNA molecule, of the present invention to be a substrate for Dicer, it has to be accessible to Dicer. The VA1 transcript is a highly structured RNA molecule that, as taught by the prior art, is not cleaved in a cell. Because it was known that the VA1 transcript is not cleaved in a cell and it was known that the RNAi

molecule has to be accessible to Dicer, it is my opinion that a skilled artisan would not have been motivated to combine the Agami et al. and Doglio et al. references, as well as to combine the Agami et al. and Doglio et al. references with the other references cited in Paragraphs 3-8. In view of the specific teachings of Doglio et al., Rossi et al. ('087) and Cagnon et al., it is my opinion that there was no reasonable expectation of success in the art that the RNAi molecule of the present invention, i.e., shRNA or precursor miRNA, would be processed from the VA1 transcript containing it so that the RNAi molecule would become a substrate for Dicer. Because there is no reasonable expectation of success, it is my opinion that the skilled artisan would not have been motivated to make the proposed combinations of prior art set forth in Paragraphs 3-8.

22. In summary, it is my opinion that the prior art specifically teaches that the VA1 transcript is not cleaved in a cell and that the VA1 transcript containing an antisense oligonucleotide or a ribozyme is not cleaved in a cell. Because of these teachings in the prior art, it is my opinion that there is no reasonable expectation of success that an shRNA or precursor miRNA produced as part of a VA1 transcript would be cleaved or processed from the VA1 transcript. Because there is no reasonable expectation of success, there is no motivation to combine the cited references as proposed by the Examiner. Because the prior art teaches that there is no cleavage of the VA1 transcript or processing of the VA1 transcript to release the antisense oligonucleotide or ribozyme, it is my opinion that the prior art teaches away from the present invention.

23. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true. All statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

May 8, 2009
Date



John J. Rossi